

CLAIMS

1. Immunoreactive HTLV-III polypeptide expressed by cells transformed with a recombinant vector containing HTLV-III cDNA.
2. A polypeptide of Claim 1 wherein said HTLV-III cDNA encodes an env gene sequence.
3. A polypeptide of Claim 2 wherein which is immunoreactive with sera of patients with acquired immunodeficiency syndrome.
4. Isolated HTLV-III envelope polypeptide.
5. Isolated cDNA encoding an HTLV-III gene.
6. cDNA of Claim 5 encoding the HTLV-III env gene.
7. Isolated cDNA encoding for an HTLV-III polypeptide which is immunoreactive.
8. Isolated cDNA of Claim 7 coding for an envelope polypeptide which is immunoreactive.
9. A DNA probe comprising a DNA sequence coding a portion of the HTLV-III genome.
10. A DNA probe of Claim 9 wherein the DNA sequence encodes at least a portion of the env gene.

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11. A hybrid protein comprising an HTLV-III polypeptide linked to at least one other polypeptide.
12. A hybrid protein of Claim 11 comprising an HTLV-III polypeptide linked to an indicator polypeptide.
13. A hybrid protein of Claim 12 wherein said indicator polypeptide comprises beta-galactosidase.
14. An isolated RNA transcript of the env gene of HTLV-III.
15. An isolated RNA transcript of Claim 14 having a label which emits a detectable signal.
16. An isolated RNA transcript of Claim 15 wherein said label comprises a radioisotope.
17. A recombinant vector containing HTLV-III DNA capable of expression upon insertion into host cells.
18. OmpA vector containing HTLV-III cDNA.
19. pMR 100 vector containing HTLV-III cDNA.
20. A method of producing HTLV-III polypeptide, comprising the steps of:
  - a. cleaving HTLV-III cDNA to produce DNA fragments;

- b. inserting the DNA fragments into an expression vector to form a recombinant vector;
- c. transforming an appropriate host cell with the recombinant vector; and
- d. culturing the transformed host cell under conditions sufficient for expression of the polypeptide coded for by the inserted HTLV-III DNA.

- 21. A method of Claim 20 wherein the cleaving step comprises digesting the HTLV-III cDNA with restriction endonucleases to produce restriction fragments of cDNA.
- 22. A method of Claim 20 wherein the cleaving step comprises shearing the HTLV-III cDNA to produce cDNA fragments.
- 23. A method of producing HTLV-III envelope polypeptide, comprising the steps of:
  - a. cleaving HTLV-III genomic cDNA with the restriction endonuclease SstI;
  - b. digesting the cleaved cDNA with restriction endonucleases sufficient to generate restriction fragments which encompass at least a portion of the env gene;
  - c. isolating the restriction fragments;
  - d. producing DNA fragments of about 200-500 base pairs in length from the restriction fragments;
  - e. isolating the DNA fragments of about 200-500 base pairs;

f. inserting the isolated fragments into the open reading frame expression vector pMR100 for production of hybrid proteins comprising an env gene product and beta-galactosidase;

g. transforming lac z<sup>-</sup> E. coli cells with the vector;

h. plating the transformed cells on MacConkey agar plates, maintaining the plates under conditions sufficient for the formation of colonies and selecting cell colonies exhibiting a red color;

i. culturing transformed cells from the selected colonies under conditions which allow expression of the hybrid protein;

j. obtaining cellular protein from the cultured transformed cells;

k. separating the cellular protein obtained;

l. contacting the separated protein with sera from AIDS patients to identify protein which is immunoreactive with the sera; and

m. isolating the immunoreactive protein.

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24. A method of Claim 23, further comprising the step of separating the env gene expression product from the remainder of the hybrid protein.

25. A fusion protein produced by the method of Claim 23.

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26. A HTLV-III envelope polypeptide produced by the method of Claim 24.
27. Antibody specifically reactive with HTLV-III envelope polypeptide.
28. An antibody of Claim 27 which is monoclonal.
29. Antibody specifically reactive with HTLV-III polypeptide produced by recombinant DNA techniques.
30. An antibody of Claim 29 which is monoclonal.
31. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III polypeptide produced by recombinant DNA techniques.
32. An immunoassay for the detection of HTLV-III employing antibody which reacts specifically with HTLV-III envelope polypeptide.
33. An immunoassay of Claim 32 wherein said antibody is monoclonal.
34. A method for detecting the presence of HTLV-III in a bodily fluid comprising the steps of:
  - a. contacting an immunoabsorbent comprising a solid phase having an antibody which specifically binds HTLV-III polypeptide with the bodily fluid;

- b. separating the immunoadsorbent and the fluid;
  - c. contacting the immunoadsorbent with a labeled antibody which specifically binds HTLV-III polypeptide; and
  - d. measuring the amount of label associated with the immunoadsorbent to determine the presence of HTLV-III.
- 35. An assay kit comprising an antibody which reacts specifically with HTLV-III polypeptide bound to a solid phase and a labeled antibody which reacts specifically HTLV-III polypeptide.
- 36. A method of determining the presence of antibodies against HTLV-III in a bodily fluid comprising the steps of:
  - a. contacting an immunoadsorbent comprising an HTLV-III polypeptide bound to a solid phase with a bodily fluid;
  - b. separating the immunoadsorbent from the bodily fluid;
  - c. contacting the immunoadsorbent with a labeled HTLV-III polypeptide; and
  - d. determining the amount of labeled polypeptide bound to immunoadsorbent as an indication of antibody to HTLV-III.
- 37. A kit for determining the presence of antibody against HTLV-III in a bodily fluid comprising:
  - a. an immunoadsorbent comprising a HTLV-III polypeptide bound to a solid phase; and

b. labeled HTLV-III polypeptide.

38. A method of detecting HTLV-III nucleic acid in a bodily fluid comprising the steps of:

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- a. adsorbing the nucleic acid in a bodily fluid onto an adsorbent;
  - b. denaturing the adsorbed nucleic acid;
  - c. contacting the adsorbed nucleic acid with a HTLV-III DNA or RNA probe; and
  - d. determining if the probe hybridizes with the adsorbed nucleic acid.

4-16 39. A method of Claim 38 wherein the bodily fluid is a cell lysate.

40. A hybridoma cell line which produces antibody specifically reactive with HTLV-III envelope polypeptide.

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